<ins>REMARKS</ins>

The present invention is drawn to penem compounds having a cis configuration, and substituted at the six-position with a 1-hydroxypropyl group. The present compounds thus have a (1'S, 5R, 6R) configuration, which is equivalent to a (5R, 6R, 8S) configuration. The claimed compounds are particularly effective against methicillin-resistant *staphylococcus aureus* (MRSA) as disclosed in the specification in the paragraph bridging pages 2 and 3.

As now amended, in the formula (I), the 2-position substituent R_1 (when not hydrogen) has been restricted to those groups bonded to the 2-position of the penem skeleton via a thio group.

As a preface to the discussion below of the rejections over prior art, Applicants submit that the following background information is highly pertinent.

As further disclosed at pages 2-3 of the specification, analogous compounds containing a 1-hydroxyethyl group as a 6-position substituent have insufficient activities compared with those having a (1'R, 5R, 6S) configuration. Moreover, it is also known that if the steric configuration of the 6-position hydroxyalkyl group is (1'R, 5R, 6S), compounds containing a propyl or a higher alkyl group as the alkyl group are no longer equipped with any substantial activity. Therefore, the art has primarily investigated substitution at the 2-position for the improvement of activities of penem compounds. Thus, against the above background, it was surprising and unexpected that the presently-claimed compounds possessed the asserted activity.

With an object of creating antibiotics having broad spectrum and high antibacterial activities against pathogenic bacteria with acquired resistance to antibiotics led by highly

resistant MRSA which has recently been increasing in number, the invention of the present application was completed as a result of determination of correlations between the kinds and steric configurations of 6-position substituents on the penem ring and biological activities. Specifically, the invention of the present application is concerned with penem antibiotics characterized by a 1'-hydroxypropyl group at the 6-position of the penem ring and a (1'S, 5R, 6R) steric configuration (hereinafter called "cis-penem propyl derivatives").

Concerning penem compounds, those containing a 1'-hydroxyethyl group as a substituent group on the 6-position of the penem ring and having a (1'R, 5R, 6S) steric configuration, in other words, trans-penem ethyl derivatives have heretofore been reported as subject of research work for the conversion of the 2-position substituent group as in the case of other β-lactam antibiotics. The reference cited by the Examiner were published generally in the 1980s. As is readily understood from the technical contents of the references, their publications took place around the same time as interest began to focus on trans-penem ethyl derivatives. Lack of subsequent reports on cis-penems is considered to clearly reflect the history that the subject of subsequent research work increasingly shifted to trans-penem ethyl derivatives.

The rejection of Claims 1, and 37-40 under 35 U.S.C. §102(b) as anticipated by EP 0069373 to Minamida et al, is respectfully traversed. The Examiner asserts that Minamida et al disclose the racemate of the presently claimed compounds. However, a racemate does not anticipate the compound, because the compounds are not claimed in combination with their enantiomer. Nor would it have been obvious to separate the 1'S from the 1'R enantiomer. Nor does it appear that any 1-hydroxypropyl compound substituted at the 6-position were

actually synthesized in Minamida et al.

Minamida et al discloses synthesis of several types of cis-penem ethyl derivatives. It however contains neither disclosure nor suggestion about a correlation between a cis configuration and activity. Concerning the 1'-hydroxypropyl group, in particular, this reference contains no indication even about its steric configuration.

Based on the species on page 11, the Examiner asserts, in effect, that the cis form is in the public domain. However, neither disclosure nor suggestion is made at all about a correlation between a cis configuration and activity. The technical disclosure of this reference is nothing more than a mere reference to certain types of compounds. This reference does not disclose any problem to be solved for the creation of the cis-penem propyl derivatives according to the invention of the present application, and does not even provide a motive toward the creation of the cis-penem propyl derivatives according to the present invention, let alone disclosure of their creation.

Nor does Minamida et al disclose a compound having the presently-recited R_1 group.

For all the above reasons, it is respectfully requested that the rejection over Minamida et al be withdrawn.

The rejection of Claims 1, 33, and 37 under 35 U.S.C. §103(a) as unpatentable over U.S. 4,540,579 to Afonso et al, is respectfully traversed. Afonso et al is no better than the above-discussed prior art, and indeed Afonso et al direct persons skilled in the art to the (5R, 6S, 8R) configuration and 1-hydroxyethyl as the 6-substituent, i.e., trans-penem ethyl derivatives. Applicants recognize that Afonso et al does disclose cis isomers within their broad disclosure, such as at column 2, line 20. However, Afonso et al neither discloses nor

suggests anything about a correlation between a cis configuration and activity. Concerning cis-penem propyl derivatives, in particular, Afonso et al makes no mention about a problem to be solved, to say nothing of means for solving this problem. Afonso et al does not provide even a motive toward the invention of the present invention application, let alone its teaching. The statute requires that the subject matter as a whole must be considered. One skilled in the art would not consider Afonso et al in a vacuum, but would bring to the consideration all prior art knowledge in this field. Armed with such knowledge, one skilled in the art would not be led to the presently claimed compounds.

Nor does Afonso et al disclose a compound having the presently-recited R_1 group.

Accordingly, it is respectfully requested that the rejection over Afonso et al be withdrawn.

The rejection of Claims 1, 17-19, 32-34, 37 and 54-56 under 35 U.S.C. §103(a) over the literature reference "Synthesis of Optically Active Penems" (Girijavallabhan et al) is respectfully traversed. Girijavallabhan et al is deficient for substantially the same reasons as Afonso et al, which reasons are hereby incorporated by reference. While compound (23) of Girijavallabhan et al does have a (1'S, 5R, 6R) configuration, that compound contains a 1-hydroxyethyl group. Compound (14), which is a trans-isomer of Compound (23), has been reported to have been subjected to a clinical test subsequently. **Submitted herewith** is a copy of a report of this test, as discussed in Mendez et al, "High-performance liquid chromatographic methods for the determination of the penems SCH 29482 and FCE 22101 in human serum and urine, " *Biomedical Applications* (1992). When this report is taken into parallel consideration, Girijavallabhan et al will be understood to indicate that researchers at

that time were interested in the 1'-hydroxyethyl group, especially the trans form and also that even the cis form did not attract researchers' interests or repelled researchers' interests, to say nothing of the 1'-hydroxypropyl group.

Accordingly, it is respectfully requested that the rejection over Girijavallabhan et al be withdrawn.

The rejection of Claims 1-34, and 37-68 under 35 U.S.C. §103(a) as unpatentable over JP 4-69387 to Ishiguro et al, is respectfully traversed. Ishiguro et al relates to a process for the preparation of cis-form penems by irradiation with light. The compounds subjected to photoirradiation are trans-penem ethyl derivatives, and Ishiguro et al contains no disclosure or suggestion whatsoever about a correlation between a cis configuration and activity. Moreover, Ishiguro et al does not disclose any problem to be solved for the creation of cis-penem propyl derivatives. Ishiguro et al does not provide even a motive toward the invention of the present application, let alone its teaching. The relevant disclosure of Ishiguro et al is no more relevant than that of the other prior art references discussed above. Again, there is no direction in Ishiguro et al to make a 1-hydroxypropyl-substituted compound at the 6-position for a (5R, 6R, 8S) penem. Accordingly, it is respectfully requested that the rejection over Ishiguro et al be withdrawn.

The rejection of Claims 1-4, 7-19, 31-34, 37-57, 60, 63 and 66-68 under U.S. 4,742,052 to Sunagawa et al, is respectfully traversed. Again, and as discussed above, Sunagawa et al is no more relevant than the above-described prior art, since the only disclosure of (5R, 6R, 8S) compounds are those substituted at the 6-position with 1-hydroxyethyl. Moreover, why would one skilled in the art be led to the (5R, 6R, 8S)

configuration, when Sunagawa et al disclose that the (5R, 6R, 8R) and (5R, 6S, 8R) configurations are most preferred? Sunagawa et al contains no disclosure or suggestion whatsoever about a correlation between a (1'S, 5R, 6R) configuration and activity. Namely, Sunagawa et al does not disclose any problem to be solved for the creation of a cis-penem propyl derivative, and does not even provide a motive toward the invention of the present application, let alone its teaching. Accordingly, it is respectfully requested that the rejection over Sunagawa et al be withdrawn.

The rejection of Claims 1, 30, 31, 33, 37, 66, and 67 under 35 U.S.C. §103(a) as unpatentable over U.S. 4,272,437 to Menard et al, is respectfully traversed. While Menard et al disclose broadly hydroxy-substituted lower alkyl groups at the 6-position, 1-hydroxyethyl is disclosed as preferred, as is the (1'R, 5R, 6S) and (1'S, 5S, 6R) disclosed as preferred. Clearly, Menard et al directs one skilled in the art away from the presently-claimed compounds. Menard et al is deficient for essentially the same reasons as the above-discussed prior art.

Nor does Menard et al disclose a compound having the presently-recited R_1 group.

Accordingly, it is respectfully requested that the rejection over Menard et al be withdrawn.

The rejection of Claims 1-32, and 37-68 under 35 U.S.C. §103(a) as unpatentable over U.S. 4,748,162 over Leanza et al, is respectfully traversed. While Leanza et al disclose individually a 1-hydroxypropyl at the 6-position and the 5R, 6R cis isomer, the reference does not disclose the two in combination. Example 4 thereof, relied on by the Examiner, contains a 2-hydroxy-2-propyl group as the 6-substituent, not the presently-required 1-hydroxypropyl

group. Moreover, in column 20 wherein relationships between steric configurations and 6-substituents are disclosed, it is only the trans-form compound (lines 13-20) that contains 1-hydroxypropyl as a preferred 6-substituent, and 1-hydroxypropyl is not included in preferred illustrative 6-substituents for cis forms. Leanza et al does not disclose any problem to be solved for the creation of a cis-penem propyl derivative, and does not even provide a motive toward the invention of the present application, let alone its teaching. For all of the above reasons, it is respectfully requested that the rejection over Leanza et al be withdrawn.

In sum, as we have described above in detail about the individual references, the references contain neither disclosure nor suggestion at all about a correlation between a cis configuration and activity. The cis form cannot therefore be considered to belong in the public domain. The references do not suggest even the structure of a cis-penem propyl derivative, let alone a description of a structure and activity for the creation of the cis-penem propyl derivative. No matter how the references are combined, a motive toward making the present invention cannot be obtained, to say nothing of a suggestion of the present invention. The present invention is therefore by no means obvious over the references no matter how they are combined.

All of the presently pending claims in this application are now believed to be in

immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to Issue.

Respectfully submitted,

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High-performance liquid chromatographic methods for the determination of the penems SCH 29482 and FCE 22101 in human serum and urine

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ABSTRACT

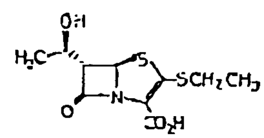
High-performance liquid chromatographic methods have been developed for the determination of two 6-(1-hydroxyethyl)penems, SCH 29482 (I) and FCE 22101 (II), in serum and urine. Serum samples were combined with an equal volume of methanol to remove proteins and, after centrifugation, an aliquot of the supernatant was analysed by ion-pair chromatography on a reversed-phase C_{18} column with hexadecyltrimethylammonium bromide as the ion-pairing agent. The compounds were detected by their ultraviolet absorbance at 305 nm for II and 322 nm for I. Urine samples were diluted, filtered and analysed by the same chromatographic procedure. At concentrations of 1–500 μg/ml of each compound, the within- and between-day precisions were 1.5–3.6 and 2.6–5.1%, respectively. The detection limit was 0.2 μg/ml for I and 0.3 μg/ml for II.

INTRODUCTION

Penems are synthetic β-lactams [1,2] characterized by their broad antibacterial spectrum and low susceptibility to hydrolysis by β-lactamases. Several agents of this class have undergone preclinical investigation. These include SCH 29482 (I), SCH 34343, FCE 22101 (II) and FCE 22891 [3–7].

The determination of β-lactam antibiotics in biological fluids is often performed by microbiological assays [8,9]. Recently, high-performance liquid chromatography (HPLC) has come into widespread use for the analytical determination of β-lactam antibiotics in biological fluids [10,11]. The speed, sensitivity and specificity of these techniques justify their use for the quantification of antimicrobial agents.

This paper presents an HPLC assay for the determination of 5*R*,6*S*,8*R*-6-(1-hydroxyethyl)-2-ethylthiopenem-3-carboxylic acid (I) and 5*R*,6*S*,8*R*-6-(1-hydroxyethyl)-2-carbamoyloxymethyl-penem-3-carboxylic acid (II, Fig. 1) in human serum and urine. It involves ion-pair reversed-


I

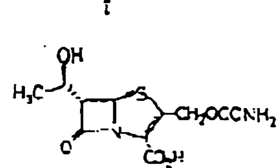

II

Fig. 1. Structures of penems: SCH 29482 (I) and FCE 22101 (II).

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phase chromatography with UV detection at 322 and 305 nm for I and II, respectively. The sample preparation procedure is simple and rapid, requiring only precipitation of protein with methanol.

EXPERIMENTAL

Reagents and materials

Compounds I and II were provided by Schering (Bloomfield, NJ, USA) and by Farmitalia Carlo Erba (Milan, Italy), respectively. Chloramphenicol was obtained from Fluka (Buchs, Switzerland). Methanol and 2-propanol were purchased from Carlo Erba. Potassium phosphate was obtained from Merck (Darmstadt, Germany), and hexadecyltrimethylammonium bromide was from Sigma (St. Louis, MO, USA). All water used in this study was purified with a Milli-Q water purification system (18 MΩ resistance) (Millipore, Bedford, MA, USA).

Ultrafiltration tubes (Ultrafree C3LGC, low binding cellulose, molecular mass cut-off 10 000) were from Nihon Millipore (Yonezawa, Japan).

Instruments

The HPLC system (Spectra-Physics SP 8800) was equipped with a variable-wavelength UV–visible detector (SP 84500) and a computing integrator (SP 4290). Samples were loaded onto the column via a Rheodyne 7125 loop injector (volume 50 μl). The column (15 cm × 3.9 mm I.D.) was of stainless steel, prepacked with 4-μm Nova-Pak C_{18} packing (Waters Chromatography Division, Millipore, Milford, MA, USA) and protected with a pre-column (30 mm × 4 mm I.D.) fitted with New Guard Cartridge RP-8 (Brownlee Labs., Santa Clara, CA, USA).

Penem standard solutions

Stock solutions of penems are prepared by dissolving 20 mg of the compounds in 10 ml of 50 m*M* phosphate buffer (pH 6.0). These stock solutions were stored at 5°C for up to two days. The 2.0 mg/ml stock solutions were further diluted 1:20 with phosphate buffer to prepare additional standards at a concentration of 100 μg/ml.

Serum and urine calibration standards

Serum and urine calibration standards were prepared by adding microlitre amounts of the 100 μg/ml or 2.0 mg/ml stock solutions to the appropriate volume of drug-free biological fluids, to produce concentrations in the range 0.5–100 μg/ml for serum and 5–500 μg/ml for urine.

Internal standard solution

Working standard solutions for the serum assay were prepared fresh on each day of analysis by dissolving amounts of chloramphenicol (internal standard) in methanol to produce concentrations of 15 and 30 μg/ml for II and I, respectively. For the urine assay the working standard solutions were prepared by dissolving the chloramphenicol in appropriate volumes of the mobile phases to produce concentrations of 10 and 15 μg/ml for II and I, respectively.

Chromatographic procedure

The chromatographic conditions are summarized in Table I. The mobile phases were prepared fresh on the day of analysis and were filtered and degassed by vacuum. All chromatographic operations were carried out at 20°C. The column was conditioned by passing the mobile phase through it for 2 h at a flow-rate of 0.5 ml/min. The detection wavelength was set at 305 nm for II and 322 nm for I.

The apparent pH values of the mobile phases used were within the pH interval where these antibiotics are most stable [12].

Sample preparation and analysis

For both antibiotics the conditions for preparation and analysis of samples were established as follows.

For serum samples, 0.5 ml of the serum calibration standard or unknown sample was pipetted into a glass tube, and 0.5 ml of working standard solution containing the internal standard was added. The tube was then vortex-mixed for 15 s and centrifuged for 15 min at 10 000 *g*. A 50-μl aliquot of the clear supernatant was loaded into the HPLC column.

For urine samples, a 0.5-ml aliquot of the urine

TABLE I

HPLC CONDITIONS FOR THE ANALYSIS OF I AND II

Parameter	Compound I	Compound II
Column type	Nova-Pak C_{18} (4 μm) (15 cm × 3.9 mm I.D.) for both antibiotics	
Flow-rate (ml/min)	0.5	0.5
Temperature (°C)	20	20
Mobile phase		
0.1 *M* Potassium phosphate (%)	75	60
Methanol (%)	–	40
2-Propanol (%)	25	–
Hexadecyltrimethylammonium bromide (ion pair) concentration (m*M*)	0.1	1.5
Apparent pH	6.20	5.75
Detection wavelength (nm)	322	305
Injection volume (μl)	50	50
Internal standard	Chloramphenicol	Chloramphenicol
Calculation	Peak area	Peak area

calibration standard or unknown sample was transferred to a 10-ml glass tube, to which 4.5 ml of working standard solution for the urine assay were added. The tubes were mixed vigorously for 15 s; 1 ml of this solution was filtered through an assembly consisting of a 0.45-μm filter (Millex-HA, Millipore) attached to a 5-ml syringe. A 50-μl aliquot of the filtrate was injected into the column.

Quantification

The serum and urine calibration standard solutions of the antibiotics were prepared at seven different concentrations between 0.5 and 100 μg/ml for serum and between 5 and 500 μg/ml for urine, and treated in the manner described above. Calibration graphs of the chromatographic peak-area ratios (antibiotic/internal standard) *versus* antibiotic concentration were constructed. Antibiotic concentrations in the unknown serum and urine samples were calculated by interpolation from the calibration graphs by a least-squares regression line treatment.

RESULTS AND DISCUSSION

Selectivity

Chromatograms from the isocratic ion-pair HPLC analysis of I and II in human serum and urine samples are shown in Figs. 2–5. Under the conditions used, I and II were well resolved from endogenous serum or urine compounds. Minor changes to the organic modifier content and/or the ion pair of the mobile phase were occasionally required to accommodate column efficiency loss, or interference from atypical serum and urine samples.

Recovery

The total recoveries of the penems were measured on blank human serum and urine spiked

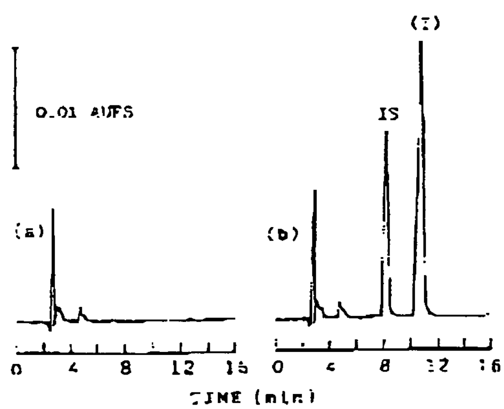

Fig. 2. Chromatograms of (a) drug-free human serum and (b) human serum spiked with 15 μg/ml SCH 29482 (I). IS = internal standard (30 μg/ml).

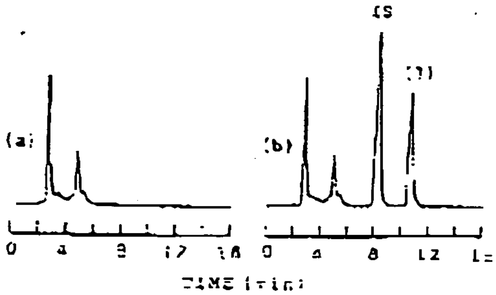

Fig. 3. Chromatograms of (a) drug-free human urine and (b) human urine spiked with 30 μg/ml SCH 29482 (I). IS = internal standard (15 μg/ml).

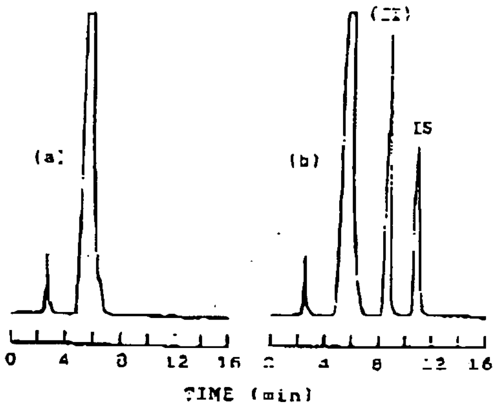

Fig. 4. Chromatograms of (a) drug-free human serum and (b) human serum spiked with 20 μg/ml FCE 22101 (II). IS = internal standard (15 μg/ml).

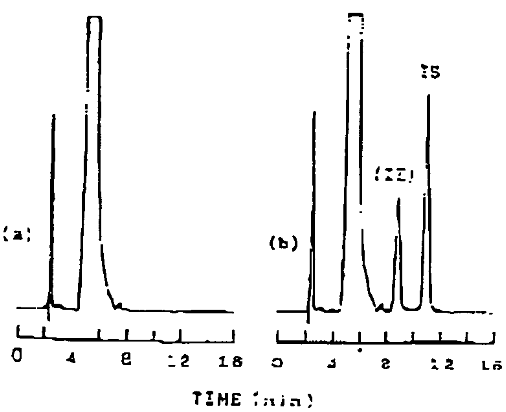

Fig. 5. Chromatograms of (a) drug-free human urine and (b) human urine spiked with 40 μg/ml FCE 22101 (II). IS = internal standard (10 μg/ml).

with these antibiotics at different concentrations. The detector responses to spiked samples were compared with those to 50 m*M* phosphate buffer solutions (pH 6.0) with identical concentrations of the compounds under study. The results (Table II) show that the recoveries were *ca.* 100%, and therefore protein binding of penems has no effect on the recoveries in serum when methanol is used to precipitate the serum proteins.

We also studied the efficacy of the ultrafiltration method for removing proteins from serum. The results showed that the main drawbacks are that *ca.* 10% of the antibiotics are absorbed in the ultrafiltration membrane, and that the method is slower and more expensive than methanol precipitation. Furthermore, the chromatograms obtained by the two methods are practically identical. When the ultrafiltration method is used, however, it is not necessary to change the precolumn as often as with methanol precipitation.

Linearity and sensitivity

For both penems we found a good linear relationship between the peak-area ratios and the penem concentrations in serum and urine samples in the range studied, with regression analysis of the data revealing a correlation coefficient of ⩽0.997 for both penems. We estimated that the limits of determination were 0.2 μg/ml for I and 0.3 μg/ml for II, with a signal-to-noise ratio of approximately 3.

TABLE II

EXTRACTION RECOVERIES OF I AND II FROM SERUM AND URINE

Sample	Concentration added (μg/ml)	Recovery (mean ± S.D.)(%)	
		Compound I	Compound II
Serum	5	99.4 ± 1.9	96.8 ± 3.3
	20	98.6 ± 3.2	99.3 ± 1.5
	50	103 ± 2.8	102 ± 3.7
Urine	5	102 ± 4.2	99.0 ± 3.1
	100	97.8 ± 2.3	97.3 ± 4.1
	500	99.3 ± 1.2	98.2 ± 2.8

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Accuracy and precision

The accuracy and precision of the assays developed for I and II in serum and urine were determined by adding known amounts of these penems to blank serum and urine. The within-day reproducibility was studied at three concentrations of I and II. To evaluate the between-day reproducibility, two concentrations of I and II were used. For both cases six serum and urine samples at each concentration were analysed by the HPLC procedure described. The results are shown in Tables III and IV, from which one can see that the coefficients of variation (C.V.) ranged from 1.8 to 5.1%, and the accuracy, defined as (amount found/amount added) × 100 (%), was *ca.* 100% for all samples assayed.

Storage stability at different temperatures

The *in vitro* storage stability in serum and urine of I and II was evaluated at −70°C, −30°C and 5°C. Freshly collected normal human serum and urine samples were supplemented with the antibiotics at concentrations of 10 and 100 μg/ml, respectively. These were assayed initially, and then stored in the dark at the stated temperatures. When aliquots of these standards were periodically analysed, I and II in serum and urine samples were stable at −70°C over the 60-day study period; however, at 5°C and −35°C, loss of both penems from serum samples was evident. The results of the analysis of these samples, given in Table V, indicate that the penems studied are especially degraded in serum samples stored at 5°C.

These results indicate that serum samples may be safely stored for up to 60 days at −70°C, and urine samples may be stored for up to 30 days at −30°C.

TABLE III

ACCURACY AND PRECISION RESULTS FOR SERUM AND URINE SAMPLES SPIKED WITH I

Concentration (mean ± S.D.) (μg/ml) Added	Found	Accuracy[a] (%)	Precision[b] (%)
Serum			
Within-day (n = 6)			
1	0.97 ± 0.03	97	3.3
25	24.2 ± 0.47	96.8	2.0
50	50.9 ± 1.43	102	2.8
Between-day (n = 6)			
5	4.97 ± 0.17	99.4	3.5
100	101 ± 2.63	101	2.6
Urine			
Within-day (n = 6)			
5	5.3 ± 0.17	106	3.3
250	246 ± 6.80	98.4	2.8
500	508 ± 14.7	101.6	2.9
Between-day (n = 6)			
5	5.1 ± 0.21	102	4.2
300	294 ± 11.2	98	3.8

[a] (Found/added) × 100.
[b] Coefficient of variation.

TABLE IV

ACCURACY AND PRECISION RESULTS FOR SERUM AND URINE SAMPLES SPIKED WITH II

Concentration (mean ± S.D.) (μg/ml) Added	Found	Accuracy[a] (%)	Precision[b] (%)
Serum			
Within-day (n = 6)			
1	1.03 ± 0.03	103	2.8
25	25.4 ± 0.59	101.6	2.3
50	50.1 ± 0.90	100.2	1.8
Between-day (n = 6)			
5	5.2 ± 0.16	104	3.0
100	100 ± 4.20	100	4.2
Urine			
Within-day (n = 6)			
5	4.8 ± 0.17	96	3.6
250	255 ± 6.88	102	2.7
500	491 ± 15.2	98.2	3.1
Between-day (n = 6)			
5	5.03 ± 0.21	100.6	4.3
300	294 ± 15.0	98	5.1

[a] (Found/added) × 100.
[b] Coefficient of variation.

TABLE V

STORAGE STABILITY DATA FOR I AND II IN HUMAN SERUM AND URINE AT 5°C AND −30°C

Storage time (days)	Found concentration (μg/ml)		C.V. (n = 3) (%)		Percentage of day zero concentration	
	5°C	−30°C	5°C	−30°C	5°C	−30°C
Compound I						
Serum						
Day zero	10.2	10.2	[illegible]	2.8	100	100
5	7.0	10.1	4.1	3.1	68.6	99
10	5.5	9.5	2.3	1.6	53.9	93.1
15	3.6	9.4	3.5	2.4	35.3	92.[illegible]
20	2.9	9.1	2.8	3.7	28.4	89.2
Urine						
Day zero	98	98	5.4	4.2	100	100
10	88	96	4.2	2.4	89.8	97.9
20	82	101	2.6	4.0	83.7	103
30	79	98	3.9	5.1	80.6	100
Compound II						
Serum						
Day zero	10.3	10.3	2.9	3.0	100	100
5	5.4	9.8	1.3	0.7	52.4	95.1
10	3.1	8.3	2.6	2.1	30	80.6
15	2.1	8.4	4.3	3.2	20.4	81.5
20	1.2	7.4	2.7	4.1	11.6	71.8
Urine						
Day zero	101	101	1.6	4.7	100	100
10	91	93	5.3	3.8	90	92
20	83	102	2.9	5.8	82.2	101
30	80	96	4.3	1.7	79.2	95

CONCLUSION

It is interesting to observe that in the penem system, in contrast with penicillins and cephalosporins, a sulphur atom is conjugated directly through a carbon–carbon double bond to the carboxylic acid and, thus, the UV spectra of penems display a long-wavelength maximum at *ca.* 310 nm. This suggests that the methods developed may be applicable to other 6-(1-hydroxyethyl)penems, with slight modifications in the mobile phase and detection at *ca.* 310 nm.

The HPLC methods developed for the quantification of I and II in human serum and urine are quick, sensitive, accurate and precise, and may be easily applied to pharmacokinetic studies of the penems in humans.

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